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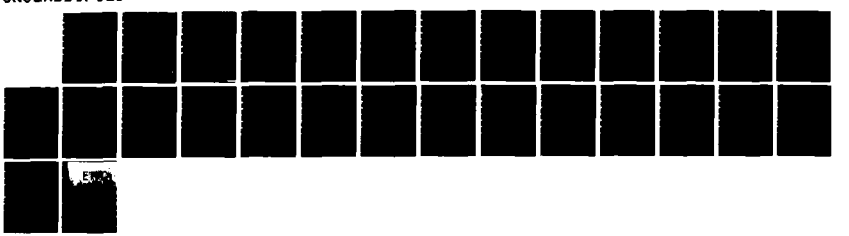

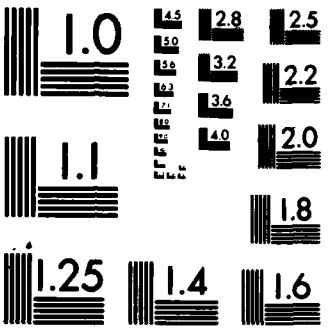


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# BLOOD PRESERVATION STUDY

## ANNUAL PROGRESS REPORT

E. Beutler

April 30, 1979

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DADA 17-70-C-0078

City of Hope National Medical Center
Duarte, Calif. 91010



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# 1. INTRODUCTION

## 1.1. Problems Addressed

The transfusion of whole blood is one of the most important factors in the dramatic improvement in the survival of wounded soldiers which has been documented in military operations since World War I. In civilian practice, too, the availability of blood and blood products has made possible feats of surgery undreamed of before blood banking became a reality during and immediately following World War II.

It was the impetus to blood banking provided by World War II which resulted in the development and shortly thereafter in the general implementation of acidified citrate solutions containing glucose for the metabolic support of the stored red cells. After collection in acid-citrate-dextrose (ACD) solution, about 70% of the red cells of whole blood or of red cell concentrates maintained their viability after 21 days of storage at $4^{o}C$. In the late 1950's relatively minor modifications of the formulation of ACD were introduced, and one of these, citrate-phosphate-dextrose (CPD) has come into common use in the past decade. The development of these preservative solutions and properties of blood and red cell concentrates collected in them has been reviewed (1-4).

Although the 21 day shelf life of red cells stored in ACD or CPD solution represents a workable system for blood storage, it is unsatisfactory in three major respects:

1.1.1. A shelf life of only 21 days makes the stockpiling of blood impossible. In civilian practice this results in recurrent blood shortages during holiday periods when demand continues high and donations decline. In contrast when collections are ample and demand may fall short of supply out-dating and wastage of blood is the consequence. In the military services, the problem is compounded by the long supply lines and therefore time required for the shipment of blood to battle zones, and by unanticipated peak demands resulting from the occurrence of military operations.

1.1.2. While blood is considered to be usable until the 21st day of storage, only about 70% of the red cells in 21 day-old units are useful to the patient. 30% of the cells are rapidly removed from the circulation and, indeed, the products of their catabolism may provide an additional strain for an already severely compromised patient.

1.1.3. The 2,3-diphosphoglycerate (2,3-DPG) content of blood stored in ACD declines rapidly after one or two days of storage, while in CPD the decline of 2,3-DPG levels is delayed for only one week. 2,3-DPG is an important modulator of the affinity of hemoglobin for oxygen (5,6). When the concentration of this compound is diminished in red blood cells tissue oxygen tensions must fall to lower levels to extract the same amount of oxygen which is extracted from normal red cells.

Extensive attempts have been made to resolve these problems by the storage of red cells at sub-freezing

temperatures. However, preservation of red cells under these circumstances requires the addition and subsequent removal of cryoprotective agents such as glycerol or dihydroxyethyl starch. This process is both expensive and cumbersome. Despite major investments of research resources in attempts to simplify the frozen storage of red cells, liquid blood storage continues, of necessity, to be the mainstay of practical blood preservation both in the civilian and the military sector.

The work performed under contract DADA 17-70-C-0078 has been directed toward resolution of the three problems associated with liquid blood storage enumerated above.

1.2. History, Performance Site and Personnel

Contract DADA 17-70-C-0078 was first implemented on November 1, 1968. The director of the project and all of the project personnel will be moving to the Scripps Clinic and Research Foundation, and application has been made to transfer the project to that institution on May 1, 1979. Ernest Beutler, M.D. has served as director of this program during its entire ten year existence. Major professional personnel participating in the studies carried out under this contract included: Lee Wood, M.D., Major Thomas Bensinger, M.C., and Ram Chillar, M.D. Technical support has been provided at various times by numerous skilled assistants, particularly by Judith Metro, B.S., Ernestine Williams and Enriqueta Guinto, B.S., Catherine Sonne, B.S., and Carol West. Blood has been obtained from over 100 paid normal volunteers and studies of red cell viability have been carried out in an additional 50 subjects. At various times during the development of new technologies under this project,

engineering and manufacturing support was obtained from McGaw Laboratories, Dow Corning, and Jet Propulsion Laboratories. The National Heart, Lung and Blood Institute provided grant support (HE 07449) for related research in red cell metabolism.

## 2. WORK PERFORMED

### 2.1. Previously Reported (Nov. 1, 1968 - Jan. 1, 1978).

#### 2.1.1. The Regeneration of Red Cell 2,3-DPG After Transfusion.

While it was generally recognized that 2,3-DPG depletion occurred in ACD or CPD-stored blood, the rate at which 2,3-DPG was restored after reinfusion was unknown. We carried out both *in vitro* (7) and *in vivo* (8) studies to provide the needed information. The *in vitro* investigations were performed by incubating 2,3-DPG-depleted erythrocytes in fresh plasma at $37^{o}$ and measuring the rise in 2,3-DPG levels. The *in vivo* studies were performed by transfusing type A patients with type O 2,3-DPG depleted erythrocytes and isolating the transfused cells by differential agglutination. These investigations indicated that approximately half of the lost 2,3-DPG was repleted in about 4 hours.

#### 2.1.2. The Effects of Composition of Storage Media on the Loss of 2,3-DPG from Stored Blood.

The most important parameter in determining the maintenance of 2,3-DPG levels during storage was found to be the pH of the blood-preservative mixture (7). Certain additives were found markedly to influence the preservation of 2,3-DPG levels. Ascorbate was found to improve 2,3-DPG maintenance (9), and this effect was particularly noticeable when the stored blood was mixed periodically (10). We found that red cells contained triokinase activity, and were able to efficiently phosphorylate dihydroxyacetone (11). Dihydroxyacetone was found to be very effective in maintaining

2,3-DPG levels (12), ascorbate and dihydroxyacetone exerted additive effects.

2.1.3. Studies of Adenine in Red Cell Preservation.

Because of the increasing proportion of red cells which are now stored as concentrates, we studied the in vitro changes during storage not only in whole blood but also in red cell concentrates stored in adenine-containing media. CPD solution was used as the basic preservative and sufficient adenine was added to provide 0.5 millimore adenine in the blood preservative mixture. Various concentrations of glucose were investigated and the depletion of ATP and 2,3-DPG from the blood or red cell concentrates were measured (13). The effect of mixing was also studied (14).

On the basis of these preliminary investigations, CPDA-1 was designed and tested in a multi-institutional study (15). The results of this investigation led to the licensing of CPD adenine for red cell preservation (see also section 2.2.1.1.).

2.1.4. Studies of the Storage of Red Cells in Artificial Preservatives.

The storage of red cells in CPD based media severely limits the conditions under which red cell storage can be investigated. For example, the addition of calcium is proscribed by the presence of the coagulation system, added magnesium would be partially chelated by the citrate which is present, and the addition of any new buffers would be attended by a prohibitive increase in the volume of the storage system. To circumvent these difficulties, we initiated investigation

of the storage of red cell concentrates in defined solutions after the citrated plasma had been removed (16,17). Bicarbonate buffer systems appeared to be particularly effective in stabilizing the pH of such stored concentrates, and it soon became apparent that the buffering effect of bicarbonate was dependent upon loss from the storage mixture of $CO_2$ formed by the interaction of bicarbonate with lactic acid formed in red cell glycolysis (18). Considerable effort has been expended in attempting to develop this finding into a practical red cell storage system. The external loss of $CO_2$ from such a system was dependent upon the surface volume ratio of the storage container and upon the gas permeability of the plastic film. Vast differences in permeability of film were found to exist. Silicone rubber, for example, was extremely permeable to $CO_2$. Various polyvinyl chloride films differed in their permeability, and polyethylene films, among others, were insufficiently permeable. Failing to find a suitable plastic film which would permit adequate $CO_2$ loss to the external environment, we turned our attention to the possibility of removing $CO_2$ from the red cell suspension by absorption into an internal $CO_2$ trap. Calcium hydroxide, enclosed in envelopes manufactured from silicone rubber film, proved to be quite satisfactory for this purpose (19). However, in order to obviate the possibility that an occasional defective bag might permit calcium hydroxide to spill into the red cell suspension, we investigated the use of silicone rubber blocks impregnated with calcium hydroxide. These, too, proved to be functionally adequate. It is of special interest that red cells stored in

the artificial medium which we have designed, BAGPM, maintained a consistently low screen filtration pressure during storage (20).

2.1.5. Other Investigations

Numerous other investigations of various aspects of red cell storage and related topics were investigated during the course of this contract. For example, to facilitate the measurement of the hydrogen ion concentration of blood at the temperature of storage, a new apparatus was designed for measurement of pH at $0^{0}$ (21). The pattern of membrane proteins during storage was investigated and no major changes were observed. Recently, this problem is being reinvestigated with the use of special techniques designed to detect small quantities of high molecular weight cross linked membrane protein complexes (see also 2.2.1.2.).

2.2. Studies not Previously Reported (Jan. 1978 through April, 1978).

2.2.1. Storage of Red Cells in CPDA-1 in PL 146

2.2.1.1. Evaluation of Whole Blood and Concentrates at Hct of 75%.

During the current contract period, we have been evaluating adequacy of CPDA-1 for storage of packed red cells and feasibility of using CPDA-2 or CPDA-3 as substitute preservatives. Studies of CPDA-1 were repeated using blood bags constructed from PL 146 film and results very similar to those obtained with the old film were obtained.

In our studies of this preservative a number of possible predictive parameters for red cell preservation were investigated. These included not only the measurement of ATP, a standard, albeit unreliable parameter of red cell viability, but also by measurement of deformability in the ektacytometer (22-25) measurement of red cell phosphofructokinase activity, agglomerability of the red cells according to the method of Meyerstein et al (26). The results of these investigations are summarized in Tables 1 and 2.

As a result of these studies and those of collaborating groups, CPDA-1 has been licensed for red cell preservation.

2.2.1.2. Viability of high hematocrit red cell concentrates prepared from CPDA-1.

Blood has been collected from normal donors into CPD or CPDA-1 and sufficient plasma removed after centrifugation to provide red cell concentrates with hematocrits ranging from 74 to 96%. Glucose, ATP, pH, and 2,3-DPG estimations have been carried out using standard techniques developed in this laboratory (27). After 21 days of storage, the red cells have been labeled with $^{51}Cr$ and reinfused into donors in order to determine the viability of the red cells. The results of these investigations are summarized in Reference 31. It is apparent that, contrary to some earlier reports (28-30), tightly packed red cells do not have adequate viability after 21 days of storage, particularly when the hematocrit of the concentrate is over 85%. CPDA-1 provides improved red cell viability, particularly when the hematocrit of the concentrate is very high. In these investigations we are

also studying the possible predictive value of high molecular weight cross-linked membrane proteins in relationship to red cell viability.

2.2.2. Glycolysis in platelet concentrates prepared from CPD, CPDA-1 and CPDA-3.

Basic studies of the effect of storage of platelets in CPD and CPD-adenine solutions have been undertaken. These include measurements of platelet glycolytic enzymes (Reference 32) of platelet metabolic intermediates during platelet storage and after incubation under physiologic conditions at storage (Reference 33), and studies of capacity of platelets to utilize citrate (Reference 34). In order to determine whether increased glucose concentration adversely affected the storageability of platelet concentrates by hastening the fall of pH, platelet concentrates were prepared in CPD, CPDA-1, and CPDA-3 in order to provide a maximum range of glucose and adenine concentrations for study. The rate of glucose consumption, lactate formation and the net accumulation of hydrogen ions was compared not only to the platelet count of the preservative solutions but also to the residual white cells which were present. The results of these studies are contained in (Reference 35). These investigations show that CPDA-3 does not have an adverse metabolic effect on platelets. Moreover, it emphasizes the important role that residual white cells may play in the storage of platelet concentrates. It is likely that the earlier results suggesting that high glucose concentrations might adversely affect platelets may have been artifactual due to increased residual numbers of leukocytes.

### 2.2.3. Studies with BAGPM

An investigational new drug application (IND) has been drafted for submission to the FDA for the institution of clinical trials with BAGPM. First, certain manufacturing problems had to be overcome, resulting in some delay in the filing of the application and implementation of studies. Fenwal Laboratories provided for us a prototype closely approximating the manufactured configuration for BAGPM. Packed red cells collected in ACD in such prototype bags were studied biochemically using various mixing schedules. The results, summarized in Table 3, indicated that the prototype was satisfactory. However, two further changes are being made because of certain manufacturing constraints. First of all, Fenwal Laboratories prefers to use CPD in the primary bag. Since the pH of CPD is higher than the pH of ACD, this might affect the pH of the final mixture sufficiently to require modification in the BAGPM formulation. It was found that the pH of the blood drawn into CPD and then stored in BAGPM is approximately 0.14 pH units higher than the pH of packed red cells from blood collected in ACD. A second alteration concerns the shape of the block. A new manufactured prototype has been submitted to us for evaluation. These prototypes contain donut-shaped calcium hydroxide blocks. This configuration apparently facilitates the production of this essential item. Our initial results with the new configuration were unsatisfactory: the pH of the red cell suspension remained too high during storage, resulting in relatively rapid decline of ATP levels and accumulation of fructose diphosphate

and triose phosphates. At first we attempted to overcome this problem by reducing the amount of sodium carbonate in the mixture so as to reduce its alkalinity. This proved to be unsatisfactory, excessively high pH values being observed even without any carbonate in the mixture. It now seems that an important consideration may be the position in which the red cell concentrate in BAGPM is stored. In studying the new configuration supplied by Fenwal, we have stored the bags in a standing position. Previous investigations had been conducted with the bags in a lying position. It now appears as though this aspect of storage has an influence on pH, probably because of the relative distance of parts of the solution from the "blockie". These studies are being pursued in order to devise the final optimal method of storage.

# BIBLIOGRAPHY


1. Beutler, E.: The maintenance of red cell function during liquid storage. Progress in Transfusion and Transplantation. Schmidt, P.J., Ed. 285-297, Washington, D.C., 1972.

2. Beutler, E.: Viability, function, and rejuvenation of liquid-stored red cells. Preservation of Red Blood Cells. Chaplin Jr., H., Jaffe, E.R., Lenfant, C. and Valeri, C.R., Eds. 195-214, National Academy of Sciences, Washington, D.C., 1973.

3. Chaplin Jr., H., Beutler, E., Collins, J.A., Giblett, E.R. and Polesky, H.F.: Current status of red-cell preservation and availability in relation to the developing national blood policy. N. Engl. J. Med. 291:68-74, 1974.

4. Beutler, E.: Experimental blood preservatives for liquid storage. The Human Red Cell in Vitro. Greenwalt, T.J. and Jamieson, G.A., Eds. 189-216, Grune and Stratton, New York, 1974.

5. Chanutin, A. and Curnish, R.R.: Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. Arch. Biochem. Biophys. 121:96-102, 1967.

6. Benesch, R. and Benesch, R.E.: The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin. Biochem. Biophys. Res. Commun. 26:162-167, 1967.

7. Beutler, E., Muel, A. and Wood, L.A.: Depletion and regeneration of 2,3-diphosphoglyceric acid in stored red blood cells. Transfusion 9:109-114, 1969.

8. Beutler, E. and Wood, L.: The in vivo regeneration of red cell 2,3-diphosphoglyceric acid (DPG) after transfusion of stored blood. J. Lab. Clin. Med. 74: 300-304, 1969.

9. Wood, L.A. and Beutler, E.: The effect of ascorbate on the maintenance of 2,3-diphosphoglycerate (2,3-DPG) in stored red cells. Br. J. Haematol. 25:611-618, 1973.

10. Wood, L.A. and Beutler, E.: The effect of periodic mixing on the preservation of 2,3-diphosphoglycerate (2,3-DPG) levels in stored blood. Blood 42:17-25, 1973.

11. Beutler, E. and Guinto, E.: Dihydroxyacetone metabolism by human erythrocytes: Demonstration of triokinase activity and its characterization. Blood 41:559-568, 1973.

12. Beutler, E. and Guinto, E.: The metabolism of dihydroxyacetone by intact erythrocytes. J. Lab. Clin. Med. 82: 534-545, 1973.

13. Bensinger, T.A., Metro, J. and Beutler, E.: In vitro metabolism of packed erythrocytes stored in CPD-adenine. Transfusion 15:135-139, 1975.

14. Bensinger, T.A., Metro, J. and Beutler, E.: The effect of agitation on in vitro metabolism of erythrocytes stored in CPD-adenine. Transfusion 15:140-143, 1975.

15. Zuck, T.F., Bensinger, T.A., Peck, C.C., Chillar, R.K., Beutler, E., Button, L.N., McCurdy, P.R., Josephson, A.M. and Greenwalt, T.J.: Report of a multi-institutional cooperative effort. Transfusion 17:374-382, 1977.

16. Wood, L. and Beutler, E.: Storage of erythrocytes in artificial media. Transfusion 11:123-133, 1971.

17. Beutler, E. and Wood, L.A.: Preservation of red cell 2,3-DPG in modified ACD solution and in experimental artificial storage media. Vox Sang. 20:403-413, 1971.

18. Beutler, E. and Wood, L.A.: Preservation of red cell 2,3-DPG and viability in bicarbonate-containing medium: The effect of blood-bag permeability. J. Lab. Clin. Med. 80:723-728, 1972.

19. Bensinger, T.A., Chillar, R. and Beutler, E.: Prolonged maintenance of 2,3-DPG in liquid blood storage: Use of an internal $CO_2$ trap to stabilize pH. J. Lab. Clin. Med. 89:498-503, 1977.

20. Chillar, R.K., Bensinger, T.A. and Beutler, E.: Maintenance of low screen filtration pressure in blood stored in a new liquid medium: BAGPM. J. Lab. Clin. Med. 89:504-508, 1977.

21. Bensinger, T.A., Metro, J. and Beutler, E.: Redesigned apparatus for anaerobic measurement of blood pH at low temperature. Am. J. Clin. Pathol. 63:264-268, 1975.

22. Bessis, M. and Mohandas, N.: Red cell structure, shapes and deformability. Br. J. Haematol. 31:5-11, 1975.

23. Bessis, M. and Mohandas, N.: Deformability of normal, shape-altered and pathological red cells. Blood Cells 1:315-321, 1975.

24. Bessis, M. and Mohandas, N.: A diffractometric method for the measurement of cellular deformability. Blood Cells 1:307-313, 1975.

25. Nakashima, K. and Beutler, E.: Effect of antispectrin antibody and ATP on deformability of resealed erythrocyte membranes. Proc. Natl. Acad. Sci. USA 75:3823-3825, 1978.

26. Meyerstein, N., Mazor, D. and Dvilansky, A.: Changes in agglomeration of human red cells in liquid storage in CPD media. Transfusion 17:115-122, 1977.

27. Beutler, E. Red Cell Metabolism. A Manual of Biochemical Methods. 2nd Ed., Grune and Stratton, New York, 1975.

28. Valeri, C.R., Szymanski, I.O. and Zaroulis, C.G.: 24-hour survival of ACD- and CPD-stored red cells. I. Evaluation of nonwashed and washed stored red cells. Vox Sang. 22:289-308, 1972.

29. Szymanski, I.O. and Valeri, C.R.: Clinical evaluation of concentrated red cells. N. Engl. J. Med. 280:281-287, 1969.

30. Gibson, J.G., Pond, J.C. and McManus, T.J.: Viability of red cells of ACD blood aseptically separated from plasma in a closed plastic system. N. Engl. J. Med. 263:634-637, 1960.

31. Beutler, E. and West, C.: The storage of "hardpacked" red blood cells in citrate-phosphate-dextrose (CPD) and CPD-adenine (CPDA-1). SO Blood. 1979 Jul. 54(1). P 280-4.

32. Tegos, C. and Beutler, E.: Platelet glycolysis in platelet storage. I. The glycolytic enzymes. Transfusion, March-April, 1979.

33. Beutler, E., Kuhl, W. and Tegos, C.: Platelet glycolysis in platelet storage. II. Levels and turnover of metabolic intermediates. SO Transfusion. 1979 Jul-Aug. 19(4). P. 467-71

34. Tegos, C. and Beutler, E.: Platelet glycolysis in platelet storage. III. The inability of platelets to utilize exogenous citrate. SO Transfusion. 1979 Sept-Oct. 19(5). P 601-3.

35. Beutler, E. and Kuhl, W.: Platelet glycolysis in platelet storage: IV. The effect of supplemental glucose and adenine. SO Transfusion. 1980 Jan-Feb. 20(1). P 97-100.

# Table 1

## CPD-II in PL-146 Blood Bag

## Packed Cells Stored 28 Days

| DONORS | | D.H. | M.F. | L.G. | J.G. |
|---|---|---|---|---|---|
| Hemoglobin (gm%) | initial | 24.70 | 23.13 | 23.28 | 23.67 |
| | final | 24.78 | 23.06 | 22.22 | 22.86 |
| Hematocrit (%) | initial | 76.5 | 73.0 | 75.25 | 75.4 |
| | final | 78.4 | 73.0 | 74.20 | 74.3 |
| Blood pH 4°C (anaerobic) | initial | 7.640 | 7.935 | 7.817 | 7.707 |
| | final | 7.115 | 7.070 | 7.108 | 7.013 |
| ATP (μMoles/gm Hb) | initial | 3.95 | 4.85 | 3.65 | 4.27 |
| | final | 2.89 | 3.30 | 2.45 | 2.37 |
| 2,3-DPG (μMoles/gm Hb) | initial | 20.57 | 15.11 | 18.86 | 17.26 |
| | final | lost | 0.116 | 0.096 | 0.30 |
| PFK (U/gm Hb) | initial | 11.38 | 9.40 | 8.50 | 7.05 |
| | final | 7.92 | 5.33 | 6.65 | 5.44 |
| Viability (%) | final | Not done | 84.5 | 75.7 | 89.1 |
| Plasma Hb (mg%) | initial | 0.75 | 1.1 | 0.95 | 0.5 |
| | final | 245 | 142.5 | 132.5 | 574 |
| Plasma Glucose (mg/dl) | initial | 500 | 465 | 440 | 460 |
| | final | 53 | 65 | 76 | 84 |
| Plasma Potassium (meq/L) | initial | 33 | 3.3 | 3.4 | 4.0 |
| | final | 84 | 62 | 77.7 | 73 |
| Plasma Sodium (meq/L) | initial | 170 | 171 | 165 | 167 |
| | final | 104 | 128 | 100 | 113 |
| Agglomeration +-disp.=positive dispersed. +-nond.=positive non-dispersed | initial | Not done | neg | neg | +-nond. |
| | final | | +-disp. | +-nond. | +-nond. |

Table 1 cont'd

CPD-II in PL-146 Blood Bag

Packed Cells Stored 35 Days

| DONORS | | E.C. | M.H. | M.K. | L.M. |
|---|---|---|---|---|---|
| Hemoglobin (gm%) | initial | 21.60 | 22.9 | 24.58 | 23.67 |
| | final | 24.26 | 22.8 | 24.58 | 23.59 |
| Hematocrit (%) | initial | 76.0 | 75.0 | 76.3 | 75.8 |
| | final | 78.0 | 74.8 | 75.8 | 76.0 |
| Blood pH | initial | 7.772 | 7.765 | 7.733 | 7.850 |
| 4°C (anaerobic) | final | 7.112 | 7.176 | 7.027 | 6.968 |
| ATP | initial | 5.60 | 4.50 | 4.38 | 4.04 |
| (μMoles/gm Hb) | final | 1.71 | 2.73 | 2.17 | 1.64 |
| 2,3-DPG | initial | 17.70 | 19.38 | 16.23 | 15.92 |
| (μMoles/gm Hb) | final | - | 0.14 | 0.03 | 0.14 |
| PFK | initial | 9.20 | 8.37 | 11.00 | 11.55 |
| (U/gm Hb) | final | 7.16 | 5.90 | 8.71 | 6.66 |
| Viability, 24 hrs (%) | final | Not done | 74.6 | 71.0 | 75.1 |
| Plasma Hb (mg%) | initial | 0.25 | 0.85 | 0.75 | 1.2 |
| | final | 340 | 513.3 | 440 | 189 |
| Plasma Glucose | initial | 480 | 480 | 490 | 480 |
| (mg/dl) | final | 11 | 46 | 31 | 25 |
| Plasma Potassium | initial | 3.1 | 3.8 | 3.3 | 3.2 |
| (meq/L) | final | 106.8 | 93.5 | 87.8 | 101 |
| Plasma Sodium | initial | 174 | 167 | 167 | 166 |
| (meq/L) | final | 82 | 88 | 94 | 95 |
| Agglomeration +-nond.-positive non-dispersed | initial | Not done | Not done | Not done | neg |
| | final | | | | +-nond. |

Table 1 cont'd

CPD-II in PL-146 Blood Bag

Whole Blood Stored 35 Days

| DONORS | | R.S. | S.C. | P.J. | A.T. |
|---|---|---|---|---|---|
| Hemoglobin (gm%) | initial | 12.23 | 13.72 | 11.34 | 10.04 |
| | final | 12.23 | 13.36 | 11.34 | 10.06 |
| Hematocrit (%) | initial | 39 | 41.3 | 35.6 | 32.0 |
| | final | 38.8 | 41.0 | 35.4 | 33.0 |
| Blood pH | initial | 7.718 | 7.753 | 7.568 | 7.610 |
| 4°C (anaerobic) | final | 7.210 | 7.228 | 7.100 | 7.080 |
| ATP | initial | 4.38 | 3.92 | 4.00 | 4.63 |
| (µMoles/gm Hb) | final | 2.81 | 1.71 | 1.74 | 2.99 |
| 2,3-DPG | initial | 18.37 | 16.80 | 13.58 | 14.63 |
| (µMoles/gm Hb) | final | 0.63 | 0.44 | 0.16 | - |
| PFK | initial | 8.86 | 10.96 | 7.08 | 11.73 |
| (U/gm Hb) | final | 6.81 | 7.91 | 6.03 | 9.43 |
| Viability, 24 hrs (%) | final | Not done | Not done | 79.1 | 83.5 |
| Plasma Hb (mg%) | initial | 26 | 33 | 4.25 | 3.9 |
| | final | 77.5 | 79.5 | 19.4 | 18.3 |
| Plasma Glucose | initial | 495 | 545 | 450 | 450 |
| (mg/dl) | final | 255 | 277 | 256 | 280 |
| Plasma Potassium | initial | 3.8 | 3.2 | 4.0 | 3.6 |
| (meq/L) | final | 27.7 | 30.9 | 25 | 21.6 |
| Plasma Sodium | initial | 173 | 172 | 166 | 167 |
| (meq/L) | final | 158 | 152 | 158 | 154 |
| Agglomeration | initial | Not done | Not done | +-disp. | +-disp. |
| +-disp.=positive dispersed. | final | | | +-disp. | +-nond. |
| +-nond.=positive non-dispersed. | | | | | |

Table 2

Ektacytometric examination of stored cells

(ratio of stressed diameter/resting diameter)

| | | 50 RPM | | 100 RPM | |
|---|---|---|---|---|---|
| | | pre-storage | post-storage | pre-storage | post-storage |
| M.F. | whole | 2.19 | 2.50 | 2.77 | 2.93 |
| 28 days | bottom 10% | 1.98 | 2.06 | 2.45 | 2.62 |
| packed cells | next 10% | 1.96 | 2.37 | 2.42 | 3.13 |
| | top 10% | 2.00 | 2.76 | 2.57 | 3.26 |
| L.G. | whole | 2.48 | 2.45 | 3.12 | 3.16 |
| 28 days | bottom 10% | 2.54 | 2.35 | 2.82 | 2.87 |
| packed cells | next 10% | 2.41 | 2.58 | 3.16 | 3.23 |
| | top 10% | 2.91 | 2.46 | 3.28 | 3.19 |
| D.H. | whole | 2.09 | 2.52 | 2.84 | 3.36 |
| 28 days | bottom 10% | 1.97 | 2.29 | 2.53 | 2.80 |
| packed cells | next 10% | 2.32 | 2.49 | 2.92 | 3.01 |
| | top 10% | 2.42 | 2.73 | 2.95 | 3.32 |
| M.H. | whole | 1.98 | 2.71 | 2.94 | 2.73 |
| 35 days | bottom 10% | 2.14 | 2.39 | 2.67 | 3.25 |
| packed cells | next 10% | 3.36 | 3.38,2.20 | 3.22 | 3.15,2.86 |
| | top 10% | 2.52 | 2.32,3.08 | 3.45 | 3.44,3.08 |

Table 2 cont'd

| | | | 50 RPM | | 100 RPM | |
|---|---|---|---|---|---|---|
| | | | pre-storage | post-storage | pre-storage | post-storage |
| M.K. | Whole | | 2.57 | 2.18 | 3.02 | 2.80 |
| 35 days | bottom | 10% | 2.14 | 2.18 | 2.59 | 2.73 |
| packed cells | next | 10% | 2.27 | 2.28 | 2.69 | 2.80 |
| | top | 10% | 2.46 | 2.52 | 3.02 | 3.11 |
| A.T. | whole | | 2.46 | 2.39 | 3.05 | 3.04 |
| 35 days | bottom | 10% | 2.17 | 2.36 | 2.72 | 2.81 |
| whole blood | next | 10% | 2.01 | 2.58 | 2.62 | 2.78 |
| | top | 10% | 2.33 | 2.55 | 2.91 | 2.95 |
| P.J. | whole | | 2.4 | 1.79 | 2.93 | 2.31 |
| 35 days | bottom | 10% | 2.36 | 1.26 | 2.85 | 1.39 |
| whole blood | next | 10% | 2.32 | 1.29 | 2.93 | 1.45 |
| | top | 10% | 2.72 | 1.96 | 3.49 | 2.53 |

Table 3: The effect of mixing on biochemical parameters of red cells stored in BAGPM with "blockies" containing 6 g $Ca(OH)_2$

| Day | | 0 | 7 | 14 | 21 | 28 | 35 | 42 | 49 |
|---|---|---|---|---|---|---|---|---|---|
| 2,3-DPG (µMoles/gm Hb) | | | | | | | | | |
| S.L. | Mixed 1 x per week | 16.34 | 13.72 | 11.90 | 8.52 | 10.83 | 7.60 | 8.05 | 8.63 |
| D.B. | | 14.33 | 15.51 | 13.10 | 15.01 | 14.64 | 9.23 | 9.55 | 9.28 |
| J.M. | | 15.73 | 16.41 | 15.43 | 12.96 | 11.03 | 9.32 | 9.17 | 7.60 |
| R.L. | Mixed 7 x per week | 17.86 | 17.72 | 14.14 | 18.45 | 20.15 | 18.13 | 20.08 | 18.09 |
| R.S. | Unmixed | 15.44 | | | | | | | 3.48 |
| ATP (µMoles/gm Hb) | | | | | | | | | |
| S.L. | Mixed 1 x per week | 3.73 | 3.48 | 4.11 | 4.71 | 3.85 | 3.61 | 3.31 | 3.45 |
| D.B. | | 4.32 | 2.66 | 2.99 | 3.27 | 2.81 | 2.77 | 2.85 | 3.05 |
| J.M. | | 4.15 | 2.65 | 2.88 | 2.61 | 2.91 | 3.02 | 2.93 | 2.99 |
| R.L. | Mixed 7 x per week | 3.63 | 1.59 | 1.40 | 1.22 | 1.09 | 1.16 | 1.03 | 0.91 |
| R.S. | Unmixed | 4.19 | | | | | | | 2.90 |
| pH ($4^{o}C$, anaerobic) | | | | | | | | | |
| S.L. | Mixed 1 x per week | 7.758 | 7.633 | 7.570 | 7.605 | 7.535 | 7.460 | 7.438 | 7.433 |
| D.B. | | 7.837 | 7.758 | 7.785 | 7.790 | 7.668 | 7.580 | 7.545 | 7.540 |
| J.M. | | 7.875 | 7.703 | 7.655 | 7.538 | 7.495 | 7.488 | 7.445 | 7.385 |
| P.L. | Mixed 7 x per week | 7.933 | 7.783 | 7.850 | 7.785 | 7.810 | 7.760 | 7.753 | 7.760 |
| R.S. | Unmixed | 7.875 | | | | | | | 7.365 |

Table 3 continued: The effect of mixing on biochemical parameters of red cells stored in BAGPM with "blockies" containing 6 g $Ca(OH)_2$

| Day | 0 | 7 | 14 | 21 | 28 | 35 | 42 | 49 |
|---|---|---|---|---|---|---|---|---|
| Supernatant Hb (mg%) | | | | | | | | |
| S.L. (Mixed 1 x per week) | | 49.0 | 51.8 | 68.5 | 90 | 100 | 120 | 146 |
| D.B. (Mixed 1 x per week) | | 18.4 | 30.5 | 40.5 | 59 | 80 | 82.5 | 79.5 |
| J.M. (Mixed 1 x per week) | 26.5 | 33.3 | 72.5 | 98 | 100 | 130 | 178 | 185 |
| R.L. Mixed 7 x per week | 12.4 | 65.0 | 62.5 | 80 | 110 | 190 | 210 | 228 |
| R.S. Unmixed | 21.7 | | | | | | | 180 |

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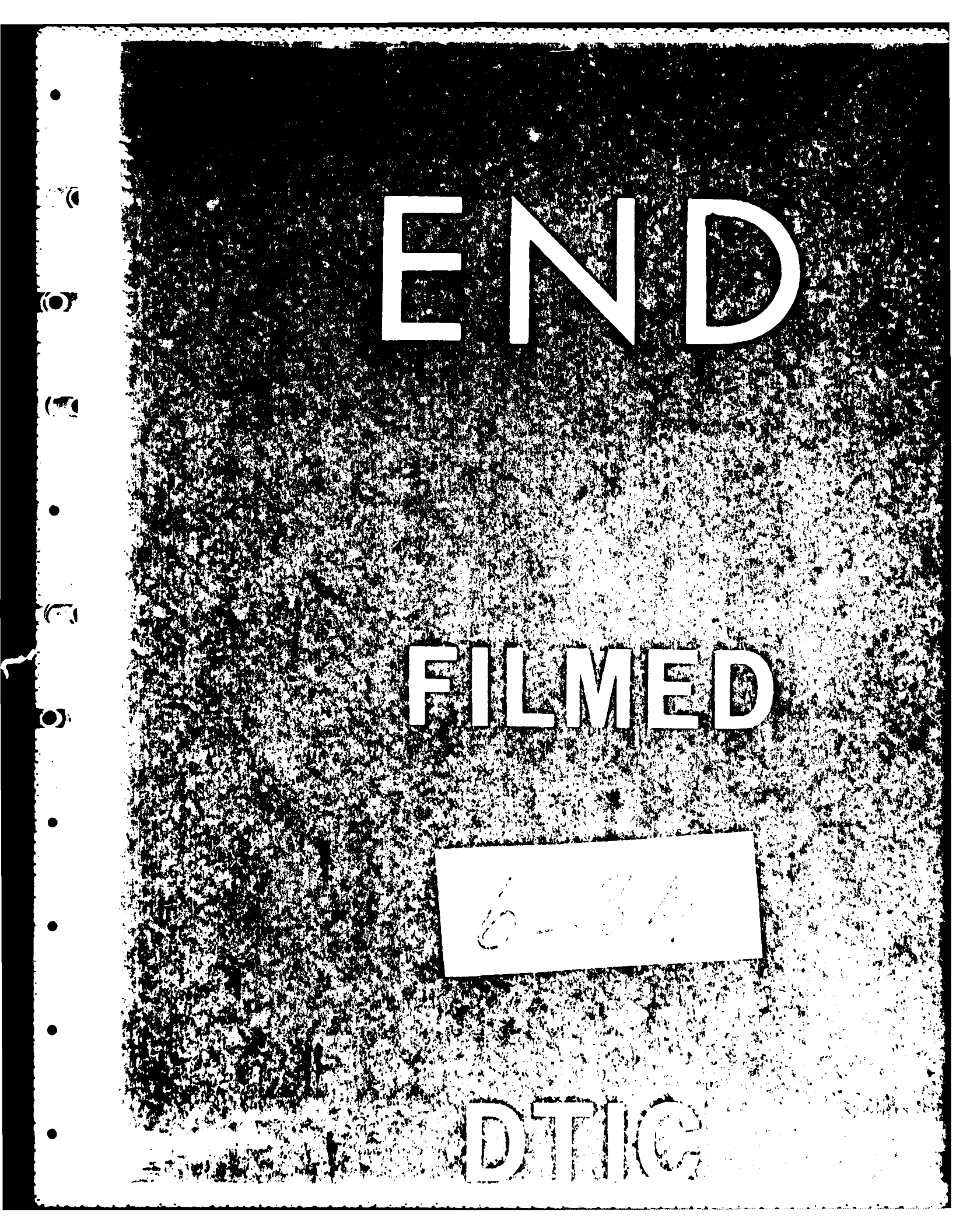
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